Library
QC
42

REGISTERED No. M 3121

PROCEEDINGS
OF THE
INDIAN ACADEMY
OF SCIENCES

VOL. XVII] *SECTION A* [No. 4

APRIL, 1943

Price Rs. 2 or 3 Sh. *Annual Subscription Rs. 18*

PRINTED AT THE BANGALORE PRESS, BANGALORE CITY, BY G. SRINIVASA RAO, SUPERINTENDENT, AND PUBLISHED BY THE INDIAN ACADEMY OF SCIENCES, BANGALORE.

SOME THEOREMS ON RESIDUES*

By P. Kesava Menon
(*Annamalai University*)

Received March 23, 1942
(Communicated by Dr. A. Narasinga Rao, F.A.Sc.)

In this paper an attempt is made to throw more light on the following statement of Hardy and Wright in their book *Introduction to the Theory of Numbers*.

"Fermat's and Wilson's theorems show that 2^{p-1} and $(p-1)!$ have the residues 1 and -1, mod p (p being prime). Little is known about their residues, mod p^2, but they can be transformed in interesting ways."

Theorems 1 to 4, under some restrictions, give such transformations for general values of the modulus m. Theorems 5 and 6 are equivalents of Gauss' lemma regarding $\left(\frac{n}{p}\right)$. The remaining theorems give results of these types.

Theorem 1. Let m, n and d be integers such that $m-1=nd$. Then

$$\frac{d^{\phi(m)}-1}{m}=\frac{1}{d}\sum_{x}\frac{\left\langle\frac{x}{n}\right\rangle}{x}, \text{ mod } m$$

where $\langle x\rangle$ denotes the smallest integer greater than or equal to x, and the summation is for all x less than and prime to m.

Proof.—Consider the array

$$\begin{array}{lll} 1 & d+1 \cdots\cdots\cdots & (n-1)d+1 \\ 2 & d+2 & (n-1)d+2 \\ 3 & \cdot & \cdot \\ \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot \\ d & 2d & nd \end{array}$$

from which all numbers not prime to $m\,[=nd+1]$ are removed. Let the product of the elements of the ith row be denoted by $\prod_i'(i+jd)$. Then

* I am thankful to the referee for some very helpful suggestions.

we have

$$\prod_j' (i+jd) = \prod_j' \{i(nd+1) - d(ni-j)\}$$

$$= \left\{\prod_j' (-d)(ni-j)\right\} \prod_j' \left\{1 - \frac{im}{d(ni-j)}\right\}$$

$$\equiv \left\{\prod_j' (-d)(ni-j)\right\} \left\{1 - \frac{im}{d} \sum_j' \frac{1}{ni-j}\right\}, \mod m^2$$

where, Σ' denotes summation over such values of $j < n$ for which $ni-j$ is prime to m. Taking the product for $i = 1, 2, 3, \ldots . d$ it is easily seen that

$$\Pi a \equiv (-d)^{\phi(m)} \Pi a \prod_{i=1}^{d} \left\{1 - \frac{im}{d} \sum_j' \frac{1}{ni-j}\right\}, \mod m^2$$

$$\equiv (-d)^{\phi(m)} \Pi a \left\{1 - \frac{m}{d} \sum_{i,j}' \frac{i}{ni-j}\right\}, \mod m^2,$$

where Πa denotes the product of all numbers less than m and prime to it.

Therefore

$$1 \equiv (-d)^{\phi(m)} \left\{1 - \frac{m}{d} \sum_{i,j}' \frac{i}{ni-j}\right\}, \mod m^2.$$

On writing $d^{\phi(m)} = 1 + \lambda m$, this gives

$$1 \equiv 1 + m \left\{\lambda - \frac{1}{d} \sum_{i,j}' \frac{i}{ni-j}\right\}, \mod m^2,$$

so that

$$\lambda \equiv \frac{1}{d} \sum_{i,j}' \frac{i}{ni-j}, \mod m$$

i.e.,

$$\frac{d^{\phi(m)} - 1}{m} = \frac{1}{d} \Sigma \frac{\left\langle \frac{x}{n} \right\rangle}{x}, \mod m.$$

Corollary 1.—If m is prime and $m - 1 = nd$, then

$$\frac{d^m - d}{m} \equiv \sum_{i=1}^{d} i \left(\frac{1}{ni} + \frac{1}{ni-1} + \ldots . + \frac{1}{ni-n+1}\right), \mod m.$$

Corollary 2.—Taking $d = 2$ in Theorem 1, which is possible if m is odd, we get

$$\frac{2^{\phi(m)} - 1}{m} \equiv \frac{1}{2} \left[\sum_j' \frac{1}{n-j} + 2 \sum_j' \frac{1}{2n-j}\right], \mod m.$$

But $\sum_j' \frac{1}{2n-j} \equiv -\sum_j' \frac{1}{n-j}, \mod m,$

so that, if m is odd,

$$\frac{2^{\phi(m)}-1}{m} = -\frac{1}{2}\sum_{j}' \frac{1}{n-j}, \text{ mod } m,$$

$$= -\frac{1}{2}\Sigma\frac{1}{x}, \text{ mod } m.$$

where Σ denotes summation over all x less than $\frac{m}{2}$ and prime to m.

Corollary 3.—Taking $d=3$ in Theorem 1, which is possible if m is of the form $3n+1$, we get

$$\frac{3^{\phi(m)}-1}{m} \equiv \frac{1}{3}\left[\sum_{j}' \frac{1}{n-j} + 2\sum_{j}' \frac{1}{2n-j} + 3\sum_{j}' \frac{1}{3n-j}\right] \text{ mod } m.$$

It is easily seen that

$$\sum_{j}' \frac{1}{3n-j} \equiv -\sum_{j}' \frac{1}{n-j}, \text{ mod } m$$

and

$$\sum_{j}' \frac{1}{2n-j} \equiv 0, \text{ mod } m.$$

Therefore

$$\frac{3^{\phi(m)}-1}{m} \equiv -\frac{2}{3}\sum_{j}' \frac{1}{n-j}, \text{ mod } m$$

$$= -\frac{2}{3}\Sigma\frac{1}{x}, \text{ mod } m$$

where m is of the form $3n+1$, and Σ denotes summation over all $x < \frac{m}{3}$ and prime to m.

THEOREM 2. Let $m+1=nd$, and $[x]$ the greatest integer $\leqslant x$. Then

$$\frac{d^{\phi(m)}-1}{m} \equiv \frac{1}{d}\Sigma\frac{\left[\frac{x}{n}\right]}{x}, \text{ mod } m.$$

This is proved exactly like Theorem 1, by observing that

$$\prod_{j}' (i+jd) = \prod_{j}' \{-i(nd-1)+d(ni+j)\}, \begin{pmatrix} i=1, 2, \ldots, d-1 \\ j<n \end{pmatrix}$$

and $\prod_{j}' (1+j)\, d = d^{\phi(m,n)} \times \prod_{j}' (j+1)$,

where $\phi(m, n)$ denotes the number of numbers not greater than n and prime to m.

Corollary.—Take $d=3$. Then

$$\frac{3^{\phi(m)}-1}{m} \equiv \frac{1}{3}\left[\sum_{j}' \frac{1}{n+j} + 2\sum_{j}' \frac{1}{2n+j}\right], \text{ mod } m.$$

But $$\sum_j{}' \frac{1}{n+j} \equiv 0, \text{ mod } m$$

and $$\sum_j{}' \frac{1}{2n+j} \equiv -\sum_j{}' \frac{1}{n-j-1}, \text{ mod } m.$$

Therefore, if m is of the form $3n-1$,

$$\frac{3^{\phi(m)}-1}{m} \equiv -\frac{2}{3}\sum_j{}' \frac{1}{n-j-1}, \text{ mod } m$$

$$= -\frac{2}{3}\Sigma\frac{1}{x}, \text{ mod } m,$$

where Σ denotes summation over all $x < \frac{m}{3}$ and prime to m.

We therefore see that Corollary 3 of Theorem 1 is true for all values of m prime to 3.

THEOREM 3. If α runs through all numbers less than m and prime to it and β through all numbers less than $\frac{m}{2}$ and prime to m, and m is odd, then

$$\Pi\alpha \equiv (-1)^{\frac{1}{2}\phi(m)} (\Pi\beta)^2 2^{2\phi(m)}, \text{ mod } m^2.$$

This can be proved in the same way as theorem 133 of Hardy and Wright's book, making use of Corollary 2 of Theorem 1 in the place of the more particular result used there.

THEOREM 4. If α, β, γ run through all numbers prime m and are such that $\alpha < m$, $\beta < \frac{2m}{3}$ and $\gamma < \frac{m}{3}$ and $\phi(m, n)$ denotes the number of numbers not greater than n and prime to m, and m is prime to 3, then

$$\Pi\alpha \equiv (-1)^{\phi(m,\, m/3)} \tfrac{1}{2}\Pi\beta \cdot \Pi\gamma \{3^{\phi(m)+1} - 1\}, \text{ mod } m^2.$$

First, let us assume that $m = 3n+1$.

Then, if Π' denotes the product for numbers prime to m, and $j < n$,

$$\prod_j{}' (1+3j) = \prod_j{}' \{(3n+1) - 3(n-j)\}$$

$$\equiv \left\{\prod_j{}' (-3)(n-j)\right\} \left\{1 - \frac{m}{3}\sum_j{}' \frac{1}{n-j}\right\}, \text{ mod } m^2$$

and $$\prod_j{}' (2+3j) = \prod_j{}' \{(m+3(n+j+1)\}$$

$$\equiv \left\{\prod_j{}' 3(n+j+1)\right\} \left\{1 - \frac{m}{3}\sum_j{}' \frac{1}{n+j+1}\right\}, \text{ mod } m^2.$$

Therefore

$$\prod_j' (1+3j) \prod_j' (2+3j) \prod_j' (3+3j) \equiv (-1)^{\phi(m,n)}\, 3^{\phi(m)} \prod_j' (n-j) \prod_j' (n+j+1) \times$$
$$\times \prod_j' (1+j) \left\{1 - \frac{m}{3}\left(\sum_j' \frac{1}{n-j} + \sum_j' \frac{1}{n+j+1}\right)\right\}, \text{ mod } m^2.$$

But $\sum_j' \frac{1}{n+j+1} \equiv 0 \text{ mod } m,$

and hence we get the required result for $m = 3n+1$ by making use of Corollary 3 of Theorem 1 and the fact that $3^{\phi(m)} \equiv 1 \text{ mod } m$.

The case $m = 3n-1$ can be treated as above, using corollary of Theorem 2.

THEOREM 5. If $m-1 = 2nd$, then $d^{\phi(m)/2} \equiv (-1)^{\nu} \text{ mod } m,$

where

$$\nu = \begin{cases} \sum_{i=1}^{n} \left\{\phi\left(m, \frac{2i-1}{2} d\right) - \phi\,(m, id)\right\} \text{ if } d \text{ is even} \\ \sum_{i=1}^{n} \left\{\phi\left(m, \frac{2i-1}{2} d - 1\right) - \phi\,(m, id)\right\} \text{ if } d \text{ is odd.} \end{cases}$$

Alternately, ν may be given by the relations

$$\nu = \begin{cases} \sum_{i=1}^{d/2} [\phi\,(m, 2ni) - \phi\,\{m, 2(i-1)n\}] \text{ if } d \text{ is even} \\ \sum_{i=1}^{(d-1)/2} [\phi\,(m, 2ni) - \phi\,\{m, 2(i-1\}n] \text{ if } d \text{ is odd.} \end{cases}$$

Proof.—Consider the array

1	$d+1$	$2d+1$	.....	$(n-1)\,d+1$
2	$d+2$	$2d+2$		$(n-1)\,d+2$
3	.	.		.
.	.	.		.
.	.	.		.
.	.	.		.
d	$2d$	$3d$		nd

from which all numbers not prime to m are removed. If we denote the product of the elements of the ith row by $\prod_j' (i+jd)$ it is clear that j runs through all values from 0 to $n-1$ such that $i+jd$ is prime to m. We have

$$\prod_j' (i+jd) = \prod_j' \{im - d(2ni-j)\}$$
$$= \prod_j' (-d)(2ni-j), \text{ mod } m.$$

Similarly

$$\prod_{j}' \{d-i+jd\} = \prod_{j}' \{d(2in+j+1)-im\}$$
$$\equiv \prod_{j}' \{d(2ni+j+1)\}, \text{ mod } m.$$

Therefore

$$\prod_{i=1}^{k} \prod_{j}' (i+jd) \prod_{i=0}^{d-k-1} \prod_{j}' (d-i+jd)$$
$$\equiv \prod_{i=1}^{k} \prod_{j}' (-d)(2ni-j) \prod_{i=0}^{d-k-1} \prod_{j}' (d)(2ni+j+1), \text{ mod } m.$$

Taking $k = d/2$ or $(d-1)/2$ according as d is even or odd we get

$$\Pi\alpha = (-1)^{\nu} d^{\phi(m)/2} \Pi\alpha, \text{ mod } m,$$

where α runs through the numbers prime to m and not greater than $m/2$. Dividing both sides by $\Pi\alpha$ we get the required result.

In a similar manner we may prove

THEOREM 6. If $m+1 = 2nd$,

$$d^{\frac{1}{2}\phi(m)} \equiv (-1)^{\mu} \text{ mod } m,$$

where $\mu = \begin{cases} \sum_{i=1}^{n} \left\{\phi(m, id-1) - \phi\left(m, \frac{2i-1}{2} d-1\right)\right\}, & \text{if } d \text{ is even} \\ \sum_{i=2}^{n} \left\{\phi(m, id-1) - \phi\left(m, \frac{2i-1}{2} d-1\right)\right\}, & \text{if } d \text{ is odd.} \end{cases}$

As corollaries of Theorems 5 and 6 we get,

THEOREM 7. If $m = 2nd+1$ be prime, then to the modulus m

$$d^{\frac{m-1}{2}} \equiv (-1)^{\frac{m-1}{4}}, \text{ if } d \text{ is even}$$
$$\equiv (-1)^{n(d-1)/2}, \text{ if } d \text{ is odd;}$$

and

THEOREM 8. If $m = 2nd-1$ be prime, then to the modulus m

$$d^{\frac{m-1}{2}} \equiv (-1)^{\frac{m+1}{4}} \text{ if } d \text{ is even}$$
$$\equiv (-1)^{n(d-1)/2}, \text{ if } d \text{ is odd.}$$

By combining the methods of Theorems 1 and 5 we get

THEOREM 9. If residues are taken to the modulus $m = 2nd+1$, and

$< x >$ and $[x]$ are as in Theorems 1 and 2.

$$\frac{(-1)^{\nu} d^{\frac{1}{2}\phi(m)} - 1}{m} \equiv \frac{1}{d} \Sigma \frac{\left\{\frac{x}{2n}\right\}}{x} \bmod m,$$

where the summation is for all $x < \frac{m}{2}$ and prime to m, and $\left\{\frac{x}{2n}\right\}$ denotes $\left\langle\frac{x}{2n}\right\rangle$ or $\left[\frac{x}{2n}\right]$ according as $\left\langle\frac{x}{n}\right\rangle$ is even or odd, and ν has the same meaning as in Theorem 5;

and

THEOREM 10. If residues are taken to the modulus $m = 2nd - 1$, then

$$\frac{(-1)^{\mu} d^{\frac{1}{2}\phi(m)} - 1}{m} \equiv \frac{1}{d} \Sigma \frac{\left[\frac{x}{2n}\right]}{x}, \bmod m,$$

where the summation is for all $x < \frac{m}{2}$ and prime to m, and $\left[\frac{x}{2n}\right]$ denotes $\left[\frac{x}{2n}\right]$ or $\left\langle\frac{x}{2n}\right\rangle$ according as $\left[\frac{x}{n}\right]$ is even or odd, and μ has the same meaning as in Theorem 6.

A TENTATIVE METHOD FOR THE ESTIMATION OF MONO-UNSATURATED AND DI-UNSATURATED GLYCERIDES

By A. R. Sukumaran Kartha and K. N. Menon
(Maharaja's College, Ernakulam)

Received March 8, 1943

A quantitative estimation of the four classes of glycerides (trisaturated, disaturated-monounsaturated, monosaturated-diunsaturated and triunsaturated) present in any fat or oil can be arrived at if any two of these two classes can be analytically determined. The trisaturated glycerides (GS_3) are capable of exact determination by permanganate oxidation in acetone, developed by Hilditch and Lea[1] arising out of Armstrong's and Hilditch's method[2] for preparing saturated esters from a mixture. The estimation of the disaturated-monounsaturated glycerides (GS_2U) has been attempted by the separation of the monoazelao-glycerides (GS_2A) formed by oxidation, as their metallic salts, by Lea[3] and others. A comparison of published data shows that when a fat yielding 25% of mono-, and 75% of diazelao-glycerides is oxidised, the separation of the monoazelao-glyceride by crystallisation of its salt leaves much to be desired from the point of accuracy. For determining the completely unsaturated glycerides, Hilditch and co-workers[4] have developed two methods. From a purely experimental standpoint the estimation of the trisaturated glycerides is the most satisfactory of all the methods so far developed.

In the most recent publication to hand Hilditch and Meara[5] discuss how far it is possible by empirical calculations, based on the proportions of the component fatty acids in a fat, to predict the general proportions of the chief mixed glycerides present. About a year back we had worked out an experimental method based on saponification values for determining GS_2U-, and GSU_2-glycerides and had incorporated the same in a thesis.[6] It is our intention to pursue the subject but in view of Hilditch's recent paper we have decided to publish the work so far accomplished.

The acidic products of oxidation in the determination of GS_3-glycerides have been utilised by us in developing our method which is really an extension of Lea's[3] attempt to estimate GS_2U-glycerides. Hilditch[7] has observed that from the ethereal solution of the products of oxidation bicarbonate removes

the aliphatic acids, triazelain, and a portion of the diazelao-glycerides, the whole of the monoazelao and some of the diazelo remaining behind in the ethereal layer. For the determination of GS_2U and GSU_2 we decided to make use of the saponification equivalents of the corresponding azelao-glycerides, GS_2A and GSA_2. If we determine the saponification equivalents of the mixture of the two and that of the diazelao-compound, which can be obtained free from admixture, we can calculate the amount of each. This is possible since we know that the saturated acids occur in the same ratio in these two types of glycerides. The equality of ratio can be proved by calculating the C_{16}- and C_{18}- acids in the two types from published results. Hilditch and Stainsby[8] separated cacao butter into three fractions by systematic crystallisation from acetone and therefrom deduced that the fat is approximately composed of 2% saturated-, 77% disaturated-, and 21% mono-saturated glycerides. This fat contains 24·3 and 35·5 respectively of C_{16}- and C_{18}-acids calculated as molar percentages. By apportioning these two acids in the disaturated glycerides we get the following values:—

$$\text{Palmitic acid } 77 \times 24{\cdot}3/59{\cdot}8 = 31{\cdot}29.$$
$$\text{Stearic acid } 77 \times 35{\cdot}5/59{\cdot}8 = 45{\cdot}71.$$

It has actually been found that these two acids are distributed in GS_2U in the ratio of 32 to 45, thereby confirming the equality of ratio.[12] We have made this calculation with the data available for a large number of fats[9] and in no case did we observe any deviation which is not within experimental error. It is possible to calculate the saponification equivalents of the glycerides with this equality of distribution of saturated acids in GSU_2 and GS_2U. The saponification equivalents of the corresponding azelao-glycerides will be a measure of the glycerides themselves.

In carrying out the estimation according to our method the following steps are involved:—

(1) The fat or oil is oxidised by acetone-permanganate and GS_3 separated.

(2) The mixture of mono-, di-, and tri-azelao-glycerides is taken up in pure ether and washed alternately with bicarbonate solution (5%, five or six times) and the extract and washings rejected. This removes the whole of the monobasic acids, triazelain and a portion of the diazelo-glycerides.

(3) The ethereal solution is now cautiously extracted alternately with 10% aqueous potassium carbonate and distilled water to extract mono- and di-azelao-glycerides. The combined extract and washings are acidified to congo-red with dilute sulphuric acid and then thoroughly extracted with ether and dried over anhydrous magnesium sulphate.

(4) The ether is removed and the residue completely freed from traces of ether under reduced pressure at a very low water-bath temperature.

(5) The saponification value of a portion of the residue is determined, this value corresponding to the S.V. of the mixture GS_2A and GSA_2.

(6) The remainder of the residue is again taken up in ether and washed alternately with bicarbonate and water. The combined washings and extract acidified with dilute sulphuric acid and the liberated pure diazelao-glycerides taken up in ether and dried.

(7) The residue obtained from this ethereal solution is used for the determination of the saponification value which is that of GSA_2.

(8) From the values from (5) and (7) and knowing the amount of saturated acids present, the values of GS_2A and GSA_2 can be determined.

That only diazelao-glycerides are removed by bicarbonate washings (see 6 above), first established by Hilditch and Salatore,[10] has been confirmed by us.

By this method we have estimated GS_2A and GSA_2 in the acidic oxidation products obtained in the trisaturated glyceride estimations[11] of the oil of *Mimusops elangi* and *Jatropha curcas*. We claim for our tentative method an accuracy within experimental errors and facility of estimation within a reasonable time.

Experimental

I. Oil of Mimusops elangi.—80 Grams of the neutral oil were oxidised. The ethereal solution of mono-, and di-azelao-glycerides was washed with bicarbonate and water. It was then dried over anhydrous magnesium sulphate and the ether completely removed, yielding 22·8 g. of residue. 1·6396 gs. of this required 20·85 c.c. of seminormal KOH corresponding to a saponification value of 356·8.

Elangi oil contains[11] 44·25 of stearic, 54·05 of palmitic and 1·7 of behenic acid (in mols.), as its saturated acid components. The theoretical S.V. for dipalmitoazelain is 304·2 and the value when the palmitic acid content is 54·05 will be $304·2 \times 54·05/100$ or 164·4. Similarly the values for distearoazelain and dibehenoazelain are 125·1 and 4·2 respectively. The sum of the three values is 293·7 and on a similar basis the sum for the diazelains is 410·4. A saponification value of 356·8 corresponds to a mixture of 46·17% of monoazelao- and 53·83% of diazelao-compounds from which follows that the weight of the monoazelao-glyceride is $22·8 \times 46·17/100$ or 10·53 g.

From the theoretical values (for S.V.) we can calculate the ratio of mono- and diazelao-glycerides corresponding to the experimental value in a different manner. The difference between the theoretical values for the two types of glycerides is 116·7 and the difference between the theoretical value for the sum of the mono-azelains and experimental value for the mixture is (356·8 less 293·7) 63·1. Hence the value for the diazelao-glyceride in the sample is 63·1 × 100/116·7 or 54·06 and for the mono-azelao-glyceride 45·94. The residue of 22·8 g. is therefore composed of 10·48 g. of monoazelao and 12·32 g. of diazelao-glyceride.

A portion of the mono-, and diazelao-glyceride mixture was dissolved in ether, washed alternately with bicarbonate and water, the extract and washings combined, exhausted with ether, and the aqueous portion acidified with dilute sulphuric acid to congo-red and the liberated diazelao-glyceride, taken up in ether, dried and the ether removed. 1·5016 g. of the residue obtained required 22·0 c.c. of seminormal KOH giving a saponification value of 410·9 as against 410·4 calculated on the basis of the distribution of the saturated acids.

The whole of the above computation is based on the non-extractability of monoazelao-glycerides by bicarbonate. This was established as follows:—

21 Grams of the mixture of the two glycerides were taken up in ether and repeatedly shaken with bicarbonate and water. The combined alkaline solution was re-extrcted with ether, added to the main ether solution, dried and the ether completely removed, yielding 15·8 g. as residue. 4·0246 Grams of this required 48·9 c.c. of seminormal KOH corresponding to a saponification value of 338·2. This corresponds to a mixture of 61·95 of mono-, and 38·05 of diazelao-glycerides, the weight of the monoazelao-glyceride in the mixture being 10·49 as against 10·53 obtained previously. This proves that no monoazelao-glyceride was extracted with bicarbonate.

II. Oil of Jatropha curcas.—120 Grams of the neutral oil were oxidised yielding a residue of 39·15 g. of mono-, and diazelao-glycerides.

The saturated acids of *Jatropha curcas*[11] consists of a mixture of 5·07% myristic, 57·74% palmitic, 35·88% stearic and 1·31% arachidic acids. The saponification value for the corresponding monoazelao-glycerides is 297·54 and for diazelao-glycerides 413·22.

12·73 Grams of the mixture were taken up in ether and a portion of the diazelao-glyceride extracted with bicarbonate, regenerated by acidification and re-extracted with ether yielding 2·48 g. of a residue. 2·2494 Grams

of this residue required 33·10 c.c. of seminormal KOH giving a saponification value of 413·0 as against the theoretical value 413·22.

The ethereal solution containing the mixture of glycerides was worked up yielding a residue of 10·25 g. 3·7994 G. of this required 48·7 c.c. seminormal KOH corresponding to 359·6 as its S.V. The ratio of the two glycerides would then be :—

The difference between the theoretical values for the two types of glycerides is 115·68 and that between the theoretical for the mono-azelao and experimental for the mixture is 62·06. From this the value for the diazelao-glyceride is 62·06 × 100/115·68 or 53·64 and its weight is 39·15 × 53·64/100 or 21·00 g. The weight of the monoazelao-glycerides is 18·15 g.

Summary

The acidic portion of the oxidation products derived in the estimation of the trisaturated glycerides is made use of in experimental determination of the mono-, and diazelao-glycerides. The method consists in determining the saponification value of the mixture of the two and that of the diazelao-glyceride which can be extracted free from the monazelao-glyceride.

REFERENCES

1. *J. C. S.*, 1927, 3106.
2. *J. S. C. I.*, 1925, **44**, 180 T.
3. *Ibid.*, 1929, **48**, 41 T.
4. *J. C. S.*, 1932, 805 ; 1937, 1767. See also *Chemical Constitution of Natural Fats*, p. 194–95.
5. *J. S. C. I.*, 1942. **61**, 117 T.
6. Thesis submitted by Kartha for the M.Sc. degree in the University of Madras (March 1942).
7. *The Chemicul Constitution of Natural Fats*, page 406.
8. *J. S. C. I.*, 1936, **55**, 95 T.
9. Reference 7—Chapters 6 and 7.
10. *J. S. C. I.*, 1933, **52**, 101 T.
11. Forthcoming publications.
12. Calculated from the molar composition of the glycerides as given in Ref. 8.

THE STRUCTURAL CHEMISTRY OF NATURALLY OCCURRING FLAVONES AND FLAVONOLS

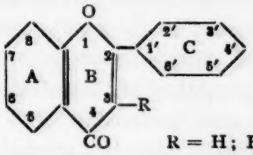
By P. Suryaprakasa Rao and T. R. Seshadri

(From the Department of Chemistry, Andhra University, Waltair)

Received March 23, 1943

Flavones and flavonols form a large group of naturally occurring organic colouring matters. Though their economic importance as dyestuffs has largely diminished, interest in their chemistry continues unabated. In connection with the important and useful investigation intended to provide a chemical basis for the botanical classification of plants, they seem to be capable of playing a part. There are other considerations also. Compounds having structures based essentially on flavones and flavonols appear to exhibit certain desirable physiological properties. For example, hesperidin has been claimed to be a vitamin and karanjin has insecticidal and other valuable properties.

Flavone ($C_{15}H_{10}O_2$) (I) the parent of this numerically large group occurs in nature. The others are structurally derived from it by the substitution of the hydrogen atoms in various ways and to varying degrees by hydroxyl or methoxyl groups. Though it may be possible for all the ten hydrogen atoms to be replaced, the maximum number of substituents so far met with is only seven. Compounds with four, five and six hydroxyl groups are more common than the others and those with five are the most numerous.



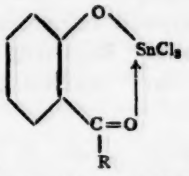
(I) R = H; Flavone
R = OH; Flavonol

For purposes of classification of flavones and flavonols the nature of the benzene ring (A) in the benzopyrone part of the molecule is taken as the basis in this paper. This ring contains four replaceable hydrogen atoms in positions 5, 6, 7 and 8. The number of hydroxyl groups in it may vary from zero to four and hence there are five main divisions. Subdivisions arise as the result of different arrangements of the groups among the four positions. From each such case a number of compounds can be derived

depending upon the condition of the side phenyl nucleus (C). So far it has been found to contain a maximum of three hydroxyl groups. Except for a solitary special case, datiscetin (which carries a hydroxyl group in 2′ position) one of these hydroxyls is invariably in the 4′ position. The others are generally found in the 3′ and 5′ positions. Morin which has two hydroxyl groups in 2′ : 4′ positions is again a special case. Consequently for each arrangement of the benzopyrone part, only four compounds arise containing 0, 1, 2 and 3 hydroxyl groups respectively in the side phenyl nucleus.

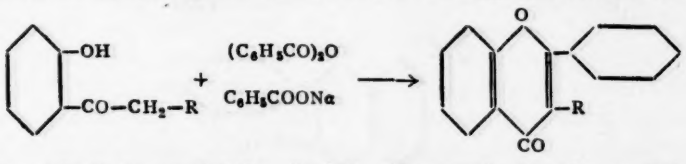
A number of useful colour reactions have been introduced in work on anthoxanthins. For detecting their presence reduction is effected by means of magnesium and hydrochloric acid or sodium amalgam in alcoholic solution.[1] Bright red or allied shades are produced and they are attributed to the formation of anthocyanins. The brightness of the colour is in general dependent upon the number of hydroxyl groups. This reaction is also given by flavanones. Amongst others giving useful information about the disposition of the hydroxyl and methoxyl groups may be mentioned the following :—(1) Boric-citric reaction of Wilson is characteristic of 5-hydroxyl or methoxyl groups.[1,2] (2) Colour of lead salts distinguishes between flavonols (red) and flavones (yelow); flavones usually give the salts only with basic lead acetate. (3) Display of bright colours[3] and exhibition of colour changes in buffer solutions of alkaline p^H and formation of brown quinones[4] when treated with *p*-benzoquinone are characteristics of flavonols having two hydroxyl groups in the para-position (5 : 8); examples are herbacetin, gossypetin, hibiscetin and cannabiscetin. (4) Bargellini's test has been considered to be specific for the compounds having hydroxyl groups in 5 : 6 : 7 positions. This is carried out by adding sodium amalgam to an alcoholic (anhydrous) solution of the compound. Green flocks are noticed: scutellarein, baicalein and quercetagetin give this test. Though it may be useful as indicating the possibility of this combination of hydroxyl groups it is not always dependable since herbacetin (5 : 7 : 8) gives a similar reaction.[5] (5) Frequently the difficulty of complete methylation of the hydroxyl groups has been taken as indication of a resistant hydroxyl in the 5 position; this arises as the result of chelation with the neighbouring carbonyl group. Diazomethane is the methylating agent used commonly for this purpose; similar results are obtained using methyl iodide and potassium carbonate or even dimethyl sulphate and alkali under ordinary conditions. This method may also be said to be only of limited applicability, since when a large excess of the reagent is used, many compounds having a 5-hydroxyl group are completely methylated. So far only flavonols with the 5 : 7 : 8

combination of hydroxyls such as herbacetin and gossypetin have resisted methylation even under these conditions. For the detection of the 5-hydroxyl group another reaction is also helpful. It is based on the observation of Pfeiffer[6] that the hydroxyl present in an ortho-position to the carbonyl group reacts with stannic chloride in benzene solution to form a substitution product of the type

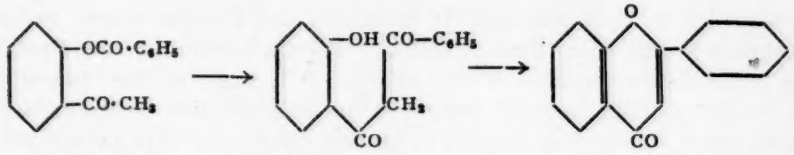


whereas, if the hydroxyl is present in any other position, double compounds of the type OH. C_6H_4.COR, $SnCl_4$ are formed. The relative proportions of tin to chlorine differ in the two cases. (6) Certain other colour reactions have been reported for the detection of hydroxyl groups situated ortho or para to each other. These are claimed to be helpful in settling the constitutions of partially methylated flavones or flavonols. Such compounds react in alkaline solutions in the cold with ortho dinitrobenzene yielding deep violet products which are considered to be quinonoid salts.[7] Compounds containing hydroxyls in ortho-positions develop an intense reddish brown colour, when a mixture of acetic acid and ammonium molybdate is added.[8] They are also said to be easily oxidised by chloropentamine cobaltichloride.[9]

For purposes of breaking down the molecules and obtaining fission products the long-established methods involving treatment with aqueous or alcoholic alkali still continue to be used. In regard to synthetic methods, however, the older ones developed by Kostanecki have been largely superseded by the more convenient method of Allan and Robinson.[10] This involves the use of the appropriate orthohydroxy acetophenone and condensing it with the anhydride and alkali salt of the appropriate aromatic acid.



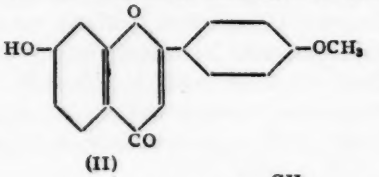
Another method also useful for obtaining flavones is that due to Baker and to Mahal and Venkataraman[11] which starts with the benzoyl derivative of the orthohydroxy ketone. This is converted under the influence of potassium carbonate or sodamide into the corresponding dibenzoyl methane which is subsequently transformed into the flavone by a suitable dehydrating agent.



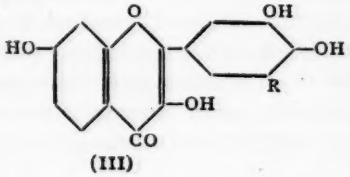
In the following brief description of the recent work in the structural chemistry of flavones and flavonols references to literature are given only in the case of recent discoveries. Regarding earlier work the well-known book of Perkin and Everest on "Natural Organic Colouring Matters" should be consulted.

I. Unsubstituted benzopyrone group.—It is remarkable that only one compound represents this group in nature and that is flavone itself (I). Derivatives with a hydroxyl in the 3 position (flavonol) or with hydroxy substituents in the side phenyl nucleus (C) do not occur. Absence of hydroxyl groups in ring (A) of the molecule is therefore accompanied by their absence all over. Flavone occurs as a characteristic dust, called 'meal' or 'farina' by gardeners, on the flower *stalks*, leaves and seed capsules of many species of the primula in an almost pure condition. In view of the fact that hydroxy flavones and flavonols are present largely in the cell sap, the occurrence of the parent compound on the outer surface of the parts mentioned above is of special interest. A similar instance is the isolation of rutin, a glycoside of quercetin from the liquid exudation found on the stalks and stems of the tomato plant.[12]

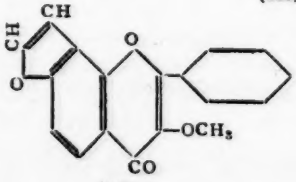
II. Monohydroxy-benzopyrone group.—



(II)



(III)



(IV)

The compounds of this group are few. Though there are four positions available for the entry of the hydroxyl, only one of these is favoured in nature and that is position 7.

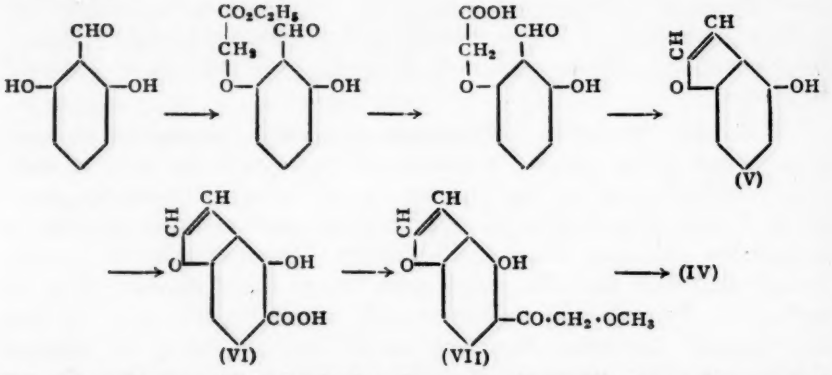
There seems to be only one flavone belonging to this group and it is pratol. It was first isolated by Power and Salway from the flowers of *Trifolium pratense* (clover) and later by Rogerson from other species of *Trifolium*. In these it occurs along with quercetin and isorhamnetin. It has the formula $C_{15}H_9O_2OH$ (OCH_3). The presence of a free hydroxyl group is indicated by its solubility in aqueous alkali to form a yellow solution and also by the formation of a monoacetate. The suggestion that its constitution should be represented as 7-hydroxy-4′-methoxyflavone (II) was made by Robinson and Venkataraman[13] who synthesised a compound of this structure from resacetophenone, anisic anhydride and sodium anisate, and found that its properties were the same as those ascribed to pratol. But direct comparison of the two could not be done as the substance from the natural source was not available.

The naturally occurring flavonols of this group are two, and they are fisetin (III, R = H) and robinetin (III, R = OH). Fisetin is present as the colouring matter of the two well-known dye woods, young fustic and yellow cedar. The former consists of the wood of the stem and larger branches of *Rhus cotinus*, a small tree which is a native of Southern Europe and the West India Islands. The latter is the wood of *Rhodosphacra rhodanthema* (common name: yellow cedar), a large tree indigenous to the northern part of New South Wales. It was synthesised by Kostanecki's method through the corresponding flavanone as well as by the easier method of Allan and Robinson.[14]

Robinetin, the higher hydroxylated compound, happens to be again a component of dye woods. It occurs as a glycoside in the wood of *Robinia pseudacacia*[15] and in the free state in the wood of *Gleditschia monosperma*.[16] It is a pentahydroxyflavone. It yields resorcinol when subjected to degradation with alkali under mild conditions. Decomposition of its pentamethyl ether with alcoholic potash gives rise to fisetol dimethyl ether (2-hydroxy-ω : 4-dimethoxyacetophenone) and trimethyl gallic acid. Schmid and Tadros[17] concluded from the above reactions, that the pigment should have the structure of 3 : 7 : 3′ : 4′ : 5′-pentahydroxy flavone (III, R = OH). It was confirmed by the synthesis of the pentamethyl ether by Brass and Kranz.[18] They condensed 4-O-methylresacetophenone with trimethylgallicaldehyde. The resulting chalkone was converted into the flavanone from which the flavonol was obtained through the isonitrosocompound. Final methylation produced the pentamethyl ether of robinetin. The flavonol itself was later obtained synthetically according to the method of Allan and Robinson by Venkataraman and his collaborators[19] and also by Charlesworth and Robinson.[20] ω-Methoxyresaceto-

phenone was condensed with trimethylgallic anhydride and the potassium or the sodium salt of the acid, when 3 : 3′ : 4′ : 5′-tetramethoxy-7-hydroxy-flavone was obtained. It yielded robinetin on demethylation and its penta-methyl ether on methylation.

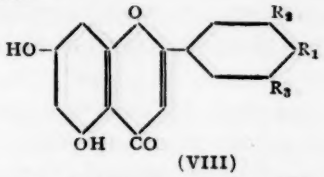
The lower members of this series of flavonols have not been found to occur as such. But karanjin (IV) isolated by Limaye from the oil of *Pongamia glabra* about twenty years ago is a derivative of 7-hydroxy-flavonol, and is probably formed from it. It is a colourless crystalline substance and is toxic to fish and smaller animals. It has the formula $C_{18}H_{12}O_4$ and shows the presence of one methoxyl group. From a study of its degradation products, karanjol (V), karanjic acid (VI) and methoxy acetyl karanjol (VII), Limaye[21] concluded that it had the constitution of 3-methoxy-7 : 8-furanoflavone (IV). This has been further supported by the work of Manjunath, Seetharamiah and Siddappa.[22] The complete synthesis of this compound has been recently worked out by Seshadri and Venkateswarlu[23] involving the following stages. Starting from γ-resorcylic aldehyde, 4-hydroxy coumarone (karanjol, V) is prepared. From this, karanjic acid (VI) and methoxy acetyl karanjol (VII) represent intermediates leading to karanjin.



The alternative synthesis beginning from 3-methoxy-7-hydroxy flavone and building up the furan ring failed at the last stage and could not be achieved.[24]

III. Dihydroxybenzopyrone group.—The two hydroxyl groups are not found in all possible arrangements. The most favoured orientation is 5 : 7. Not only are the 5 : 7 derivatives large in number but they are also very widely distributed. Recently two compounds have been obtained with the hydroxyl groups in 5 : 8 positions. The 5 : 6 combination has been found in one case.

(*a*) 5 : 7-*Combination* :—

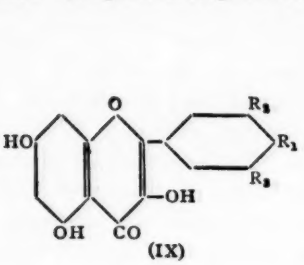


(VIII)

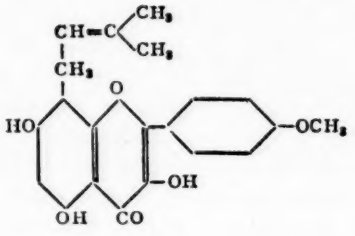
Among flavones the first three members are chrysin ($R_1 = R_2 = R_3 = H$), apigenin ($R_2 = R_3 = H$; $R_1 = OH$) and luteolin ($R_3 = H$; $R_1 = R_2 = OH$). Chrysin occurs free in the leaf-buds of the poplar and in mallows. It has recently been shown to be present in the bark of *Oroxylum indicum*.[25] Its synthesis according to the method of Allan and Robinson has been effected by Robinson and Venkataraman.[13] Apigenin is found as its glycoside in the leaves, stems and seeds of parsley and in chamomile flowers. It is also present in the flowers of *Zinnia elegans*.[26] Its 4′-methyl ether is acacetin which is present as a glycoside in the leaves of *Robinia pseudacacia*.[27] The synthesis of this ether was effected by Robinson and Venkataraman[13] by the condensation of phloracetophenone with anisic anhydride and sodium anisate. A derivative of apigenin which has been discovered more recently by Nakao and Tseng[28] from the flowers of *Daphne genkwa* is genkwanin. It is a monomethyl ether with the methoxyl in the 7 position. This was established by its synthesis by Tseng[29] starting from phloracetophenone. 4′-Benzyl ether of apigenin was first made and subjected to partial methylaüon. The monomethyl ether thus obtained was debenzylated to give genkwanin. The synthesis was also effected in a different way by Mahal and Venkataraman.[30] 4′-Benzyloxy-5 : 7-dimethoxy flavone was first made and was then subjected to debenzylation with hydrochloric acid and partial demethylation with aluminium chloride.

Luteolin is the colouring matter of the weld and of *Genista tinctoria*, and it is also present as a glycoside in the seeds of *Galega officinalis*[31] and in the flowers of *Chrysanthemum indicum*.[32] Its occurrence as a glucoside along with the glucosides of kæmpferol and quercetin in the stems of *Equisetum arvense* has also been recently reported by Nakamura and Hukuti.[33] Besides its synthesis by Kostanecki *et al.*, Lovecy, Robinson and Sugasawa[34] have also prepared it by the newer method of Allan and Robinson. Diosmetin which is present as a rhamnoglycoside in *Scrophularia nodusa* and also in many other plants was shown by Oesterle and Wander to be 4′-methyl ether of luteolin.[35] This conclusion was later confirmed by the synthesis carried out by Lovecy, Robinson and Sugasawa[34] using phloracetophenone and the anhydride and sodium salt of O-benzyl isovanillic acid, and subsequently debenzylating.

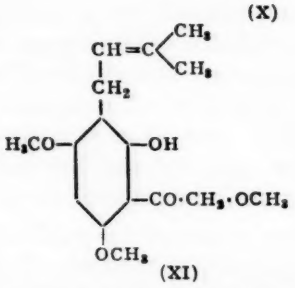
The last member of this series is tricetin (VIII, $R_1 = R_2 = R_3 = OH$) which does not occur free. Its dimethyl ether, tricin is present in the leaves of several varieties of wheat. It was first isolated by Anderson and Perkin[36] from the khapli variety, *Triticum dicoccum*. The pentamethyl ether yielded trimethyl gallic acid and 2-hydroxy-4: 6-dimethoxy acetophenone when decomposed with alcoholic potash. Anderson[37] therefore concluded that tricetin was 5: 7: 3′: 4′: 5′-pentahydroxyflavone and he obtained it synthetically by condensing phloracetophenone with trimethyl gallic anhydride and sodium trimethyl gallate and demethylating the methoxyflavone so formed. Tricin was proved to be the 3′: 5′ dimethyl ether (VIII, $R_1 =$ OH, $R_2 = R_3 = OCH_3$) by its synthesis by Gulati and Venkataraman[38] employing the anhydride and sodium salt of O-benzylsyringic acid for condensation with phloracetophenone.



(IX)



(X)



(XI)

The flavonol series is complete and the members are galangin, kæmpferol, quercetin and myricetin. The simplest compound, galangin (IX, $R_1 = R_2 = R_3 = H$) is present as a glycoside in the galanga root and is accompanied by its 3-methyl ether. It is also found in the roots of *Datisca cannabina*.[39] Besides its original synthesis by Kostanecki and Tambor, it has been prepared by Robinson and his collaborators.[40] Its 7-methyl ether is known as izalpinin, and it has recently been isolated from the seeds of *Alpinia japonica* by Kimura and Hoshi.[41] The constitution of this substance has been based upon the following considerations. It yields galangin on demethylation and is different from galangin 3-methyl ether.

Mythylation under ordinary conditions yields a monomethyl ether, which gives reactions for a free hydroxyl in 5 position. The dimethyl ether of izalpinin has been found to be identical with a synthetic sample of 3: 5: 7-trimethoxy flavone.

Kæmpferol (IX, $R_2 = R_3 = H$; $R_1 = OH$) is more abundant and occurs in the form of its glycoside in the leaves of Java indigo (*Indigofera arrecta*), in the senna leaves, in the blue flowers of *Delphinium consolida*, and also in the flowers of *Robinia pseudacacia*.[42] A diglucoside of this flavonol has been recently reported by Nakamura and Hukuti to be present in the stems of *Equisetum arvense*.[33] According to the general method, Robinson and Shinoda synthesised this compound by condensing ω-methoxyphloracetophenone, anisic anhydride and sodium anisate, and subsequently demethylating the product.[43] Its 4′-methyl ether, kæmpferide which accompanies galangin and its methyl ether in the galanga root was produced synthetically by Heap and Robinson.[40b]

An interesting derivative of kæmpferol is anhydroicaritin[44] which occurs in the leaves and roots of *Epimedium·macranthum* in the form of complex glycosides. Its constitution as 8-γ: γ-dimethylallyl-kaempferide (X) was settled mainly from (1) its oxidation with permanganate whereby acetone and anisic acid were formed and (2) the decomposition of its trimethyl ether with alcoholic potash producing the same acid along with the ketone anhydroicaritol (XI). The synthesis of this ketone was effected by Akai and Matsukawa by the condensation of 2-hydroxy-4: 6: ω-trimethoxyacetophenone with γ: γ-dimethyl-allylbromide and this was subsequently converted into trimethyl anhydroicaritin by heating with anisic anhydride and sodium anisate. This and karanjin may be considered to be the flavone analogues of the well-known insecticides of the rotenone group which are based on the isoflavone model.

Quercetin (IX, $R_1 = R_2 = OH$; $R_3 = H$) is far more important economically; it occurs in large amounts and is the most widely distributed of anthoxanthins. For purposes of dyeing it used to be obtained in large amounts from the quercitron (oak) bark in which it occurs as the glycoside quercitrin. Amongst the other sources may be mentioned the following flowers as the most important: clover, cotton, *Sophora japonica*. Its glycosides are many and varied in nature. Four methyl ethers of this flavonol also occur as glycosides. They are (1) rhamnetin (7-methyl ether) present in Persian berries and other Rhamnus species, (2) isorhamnetin (3′-methyl ether) found in the wall-flower and also in the Indian dye " Asbarg " prepared from the flowers and flowering stems of *Delphinium zalil*, (3) rhamnazin (3′: 7-

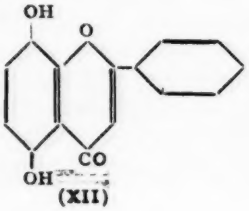
dimethyl ether) present in the Persian berries, and (4) 4′-methyl ether. The glucoside of this last substance has been recently isolated by Desai and Ahmad from the flowers of *Thevetia nerifolia*.[45] Besides the synthesis of the parent flavonol by Allan and Robinson,[14] isorhamnetin has also been prepared by Heap and Robinson using a slight modification of the general method.[40b]

The highest member of this group is myricetin (IX, $R_1 = R_2 = R_3 = OH$). It is present in considerable amounts in the bark of *Myrica nagi* and in the leaves of the Rhus species. Its presence in Venetian sumach which consists of the leaves of *Rhus cotinus* is interesting, because the wood of this constitutes "young fustic" and contains fisetin. Applying Allan and Robinson's method the flavonol was synthesised by Kalff and Robinson.[40a]

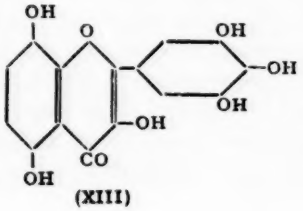
To this group of compounds having hydroxyl groups in 5:7 positions belong the two peculiar substances carrying a hydroxyl in the 2′ position. Both are flavonols. Datiscetin (5:7:2′-trihydroxyflavonol) occurs as glycoside in the leaves and roots of *Datisca cannabina*[46] (bastard hemp). Its constitution was established from the fact that on fusion with potash it yielded phloroglucinol and salicylic acid. The first synthesis carried out by Bargellini and Peratoner was not conclusive. More recently this work was done by Kalff and Robinson[39] by condensing ω-methoxyphloracetophenone with the anhydride and sodium salt of salicylic acid methyl ether and subsequently effecting demethylation.

Morin is an isomer of quercetin and has the constitution of 5:7:2′:4′-tetrahydroxy flavonol. It is the colouring matter of the dye wood, "old fustic" (*Chlorophora tinctoria*) in which it occurs as its glycoside and also of the jak-wood (*Artocarpus integrifolia*). Its pentamethyl ether on decomposition with alcoholic potash yields methoxyfisetol dimethyl ether and dimethyl ether of β-resorcylic acid. When treated with boric acid in sulphuric acid solution it exhibits a bright yellowish green fluorescence and this morin reaction is used in analytical work. It was obtained synthetically by Robinson and Venkataraman.[47]

(*b*) 5:8-*Combination*.—This combination seems to be rare. So far only one flavone, primetin and one flavonol, cannabiscetin are known and their discovery is recent.

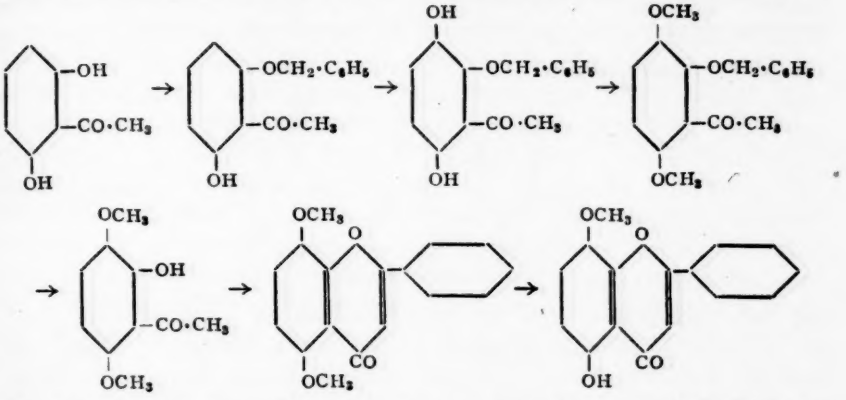


(XII)



(XIII)

Primetin ($C_{16}H_{10}O_6$) was isolated by Nagai and Hattori[48] from the leaves of *Primula modesta*. It is a dihydroxy-flavone yielding benzoic acid as the only recognisable product of hydrolytic fission. Its alcoholic solution gives a green colour with ferric chloride. One of its hydroxyl groups is difficult to methylate and hence it should be in the 5 position. From a comparison of the absorption spectra of its derivatives with those of 6-hydroxyflavone the above authors gave it the constitution of 5:6-dihydroxyflavone. This conclusion was shown to be wrong by Baker[49] who synthesised 5:6-dihydroxyflavone and found that it was different from primetin. Amongst other alternatives 5:7-dihydroxy flavone is chrysin which also differs from primetin. Consequently it was concluded to be the 5:8 dihydroxy compound (XII) and this constitution was confirmed by the synthesis of the dimethyl and monomethyl ethers by Baker *et al.*,[50] according to the following scheme:—

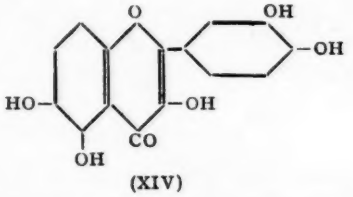


Partial demethylation of the dimethyl ether took place with aluminium chloride to yield monomethyl primetin which was identical with the compound obtained by partial methylation of primetin. Complete demethylation with hydriodic acid failed since it was accompanied by isomeric change to 5:6-dihydroxy flavone. Similar results were also obtained by Horii[51] about the same time who used for the synthesis 3:6-dimethoxy-2-benzoyloxy acetophenone and adopted Baker and Venkataraman's method of flavone synthesis.

Nakazawa[52] was successful in obtaining primetin itself synthetically by employing 2:3-dihydroxy-6-methoxy-acetophenone and condensing it with benzoic anhydride and sodium benzoate. Thus he obtained 5-methoxy-8-hydroxy-flavone which underwent smooth demethylation with aluminium chloride to yield primetin (XII).

Cannabiscetin (XIII), the only flavonol of this group, was isolated from the flowers of *Hibiscus cannabinus* by Neelakantam, Rao and Seshadri.[53] It has the formula $C_{15}H_{10}O_8$ and contains six hydroxyl groups. That it is a flavonol is clear from the readiness with which it undergoes oxidation in alkaline solution. When decomposed with alkali the hexamethyl ether yields trimethyl gallic acid. Thus four of the six hydroxyl groups of cannabiscetin could be definitely allotted to the positions 3, 3′, 4′ and 5′. The orientation of the other two as 5:8 has been arrived at from the following considerations: (1) cannabiscetin and its hexamethyl ether give positive test with boric-citric acid mixture, (2) the flavonol resembles other flavonols having hydroxyl groups in 5:8 positions, like gossypetin and herbacetin, in exhibiting marked colour changes in alkaline buffer solutions and (3) it forms a brown quinone similar to gossypetone when treated with *p*-benzoquinone. It has, therefore, been represented as 5:8:3′:4′:5′-pentahydroxyflavonol. Synthetic work in order to verify this conclusion is in progress.

(*c*) 5:6-*Combination*:

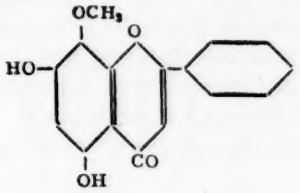


(XIV)

This very rare combination has been unknown till quite recently. Even now patuletin is the only representative and it was isolated by Rao and Seshadri[54] from the flowers of *Tagetes patula*. It is a pentahydroxy compound ($C_{15}H_{10}O_7$) isomeric with quercetin and herbacetin. It is easily oxidised by alkali and air in the cold indicating that it is a flavonol. Since protocatechuic acid could be identified as a product of this reaction, the compound is concluded to have two hydroxyl groups in 3′ and 4′ positions. Of the remaining two, one is allotted to position 5 since patuletin and its methyl ether give positive reaction with Wilson's boric-citric acid test. As it is not identical with quercetin, the 5:7 arrangement is excluded. In its reactions with alkaline buffer solutions and with *p*-benzoquinone it differs markedly from cannabiscetin and hence the 5:8 orientation too is not possible. The only other alternative is 5:6 combination and this is supported by the similarity which it exhibits to quercetagetin in regard to colour reactions and Bargellini's test. Patuletin has therefore been given the constitution of 5:6:3′:4′-tetrahydroxyflavonol (XIV). Work is in progress with a view to synthesise the compound and thus confirm its constitution.

IV. Trihydroxybenzopyrone group.—Of the four possible distributions of the hydroxyl groups, two are known to occur and they have a number of representative compounds. They are 5 : 7 : 8 and 5 : 6 : 7 combinations.

(*a*) 5 : 7 : 8-*Combination* :

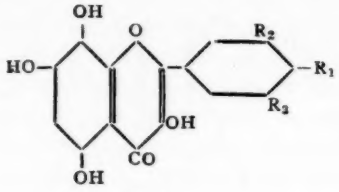


(XV)

Wogonin is the simplest flavone of this group. It is present in the roots of *Scutellaria baicalensis* along with baicalein and was first isolated by Takahashi.[55] Its constitution was first investigated by Shibata, Iwata and Nakamura[55] and later by Hattori.[56] It is a dihydroxy-monomethoxy flavone having the formula $C_{16}H_{12}O_5$. By alkaline fission benzoic acid was produced. It was therefore clear that the side phenyl ring carried no hydroxyl group. Since wogonin gave a green colour with ferric chloride and a hydroxyl was difficult to methylate, one of the two hydroxyl groups should be in position 5. Its dimethyl ether was different from trimethyl baicalein (5 : 6 : 7-trimethoxyflavone) and consequently it was considered to be a derivative of the 5 : 7 : 8-trihydroxy compound. From a study of the absorption spectrum, Hattori fixed the position of the methoxyl group at 8 and hence concluded that wogonin should be 5 : 7-dihydroxy-8-methoxy flavone (XV). This was partly confirmed by the syntheis of the dimethyl ether of wogonin by Hattori[57] himself who condensed 2-hydroxy-3 : 4 : 6-trimethoxy acetophenone with benzoic anhydride and sodium benzoate. Wogonin itself was obtained synthetically by Shah, Mehta and Wheeler[58] and the final details of the constitution were established by them. Starting from 2 : 4-dihydroxy-3 : 6-dimethoxyacetophenone, they first prepared 7-hydroxy-5 : 8-dimethoxy flavone which yielded wogonin on demethylation with aluminium chloride in nitrobenzene solution.

Populnetin seems to be the next higher member with a hydroxyl group in the side phenyl nucleus. It was discovered by Neelakantam, Rao and Seshadri[59] in the flowers of *Thespasia populnea*. It occurs in this source mostly as a glucoside and to a small extent free and is accompanied by herbacetin. It has the formula $C_{15}H_{10}O_6$ and contains four hydroxyl groups. In alkaline solution it is stable to oxidation by atmospheric oxygen and this property is very useful for separating it from the accompanying

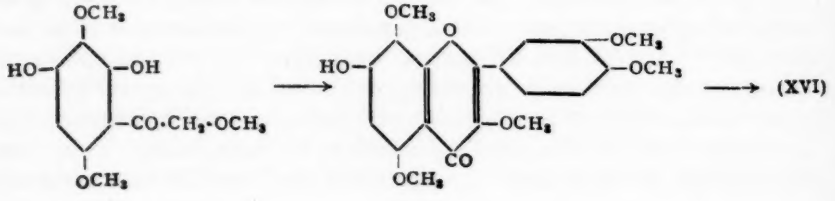
flavonols. On fusion with alkali it gives rise to *p*-hydroxybenzoic acid. Consequently a hydroxyl group should be in the side phenyl nucleus (C) in the 4′ position. The disposition of the other three hydroxyl groups appears to be 5 : 7 : 8 by analogy with herbacetin with which it occurs in the *T. populnea* flowers.



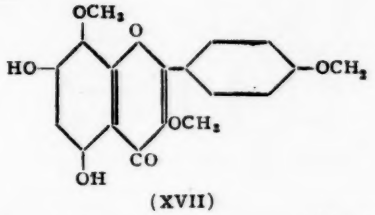
(XVI)

Three out of the possible four flavonols of this group are now known to occur in nature. They are herbacetin, gossypetin and hibiscetin and they exhibit in common the following important characteristics: (1) they produce prominent colour changes in buffer solutions of p_H on the alkaline side, (2) they form brown *p*-quinones (gossypetone reaction) readily by the action of *p*-benzoquinone, (3) the hydroxyl group in the 5th position is definitely resistant to methylation even with a large excess of diazomethane.

Of the three, gossypetin (XVI, $R_1 = R_2 = OH$; $R_3 = H$) was the earliest to be discovered. It was isolated from the flowers of the Indian and Egyptian cotton and studied in detail by Perkin. It is a pentahydroxy flavonol giving by oxidative fission in alkali protocatechuic acid. When its hexamethyl ether was hydrolysed with alkali, veratric acid and a ketonic part, gossypetol tetramethyl ether were produced. It was, therefore, considered by Perkin to be a hydroxy-quercetin with three hydroxyl groups in the benzene ring (A). The exact orientation was, however, established by its synthesis by Baker, Nodzu and Robinson.[3a] It involved the initial preparation of 2 : 4-dihydroxy-3 : 6 : ω-trimethoxyacetophenone and subsequent condensation with the anhydride and sodium salt of veratric acid. Final demethylation yielded gossypetin.



Herbacetin (XVI, $R_1 = OH$; $R_2 = R_3 = H$), the lower member of the series was obtained by Neelakantam and Seshadri[3b] from the Indian cotton flowers (*Gossypium herbaceum* and *G. indicum*). Later it was found to be present in the flowers of *Thespasia populnea* by Rao and Reddi.[60] It has the formula $C_{15}H_{10}O_7$ and contains five hydroxyl groups. In alkaline solutions it is readily broken down and *p*-hydroxybenzoic acid is formed. It closely resembles gossypetin in all its reactions and consequently it was represented by Neelakantam and Seshadri as 5 : 7 : 8 : 4′-tetrahydroxy flavonol. This constitution was confirmed synthetically by Goldsworthy and Robinson[61] who obtained it by following the procedure that was adopted earlier for gossypetin.



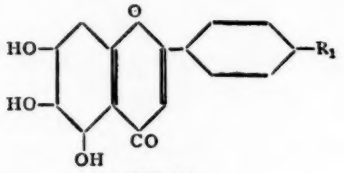
(XVII)

Herbacetin structure seems to occur more widely than was originally anticipated. A partially methylated derivative of it is found in the fruits of *Zanthoxylum acanthopodium*. It was isolated by Bose and Bose[62] and was named tambulin. It is a trimethoxy-dihydroxy compound and its relationship to herbacetin was established from the fact that on methylation the pentamethyl ether of herbacetin is obtained. The orientation of the methoxyl groups was settled from the following considerations: (1) the compound is stable to oxidation by air in alkaline solution and hence there is a methoxyl in the 3 position; (2) on boiling with alcoholic potash, anisic acid is obtained and this fixes up another methoxyl group in the 4′ position; (3) its other reactions indicate that two hydroxyl groups do not exist ortho or para to each other and therefore the third methoxyl should be in the 8 position. All these conditions are satisfied by giving tambulin the constitution of 3 : 8 : 4′-O-trimethyl herbacetin (XVII).

Hibiscetin, the third member of this series, is one of the most highly hydroxylated compounds among flavones and flavonols. Its occurrence along with gossypetin in the flowers of *Hibiscus sabdariffa* was noted by Perkin.[63] Recently these flowers have been studied in detail by Rao and Seshadri[3c] who observed that this flavonol occurs in the form of its glycoside, hibiscitrin as the major component of the colouring matter of the flowers. They have proposed for it the constitution of 5: 7: 8: 3′: 4′: 5′-hexahydroxyflavonol (XVI, $R_1 = R_2 = R_3 = OH$) from the following considerations.

(1) It has the formula $C_{15}H_{10}O_9$ and gets readily oxidised in alkaline solutions. (2) When its heptamethyl ether is decomposed with alcoholic potash trimethyl gallic acid is formed. (3) In its reactions with alkaline buffer solutions and with *p*-benzoquinone it closely resembles gossypetin and herbacetin. This structure has been confirmed by the synthesis of the methyl ether by Rao[64] who has condensed 2:4-dihydroxy-3:6:ω-trimethoxy acetophenone with the anhydride and sodium salt of trimethyl gallic acid and then methylated the product.

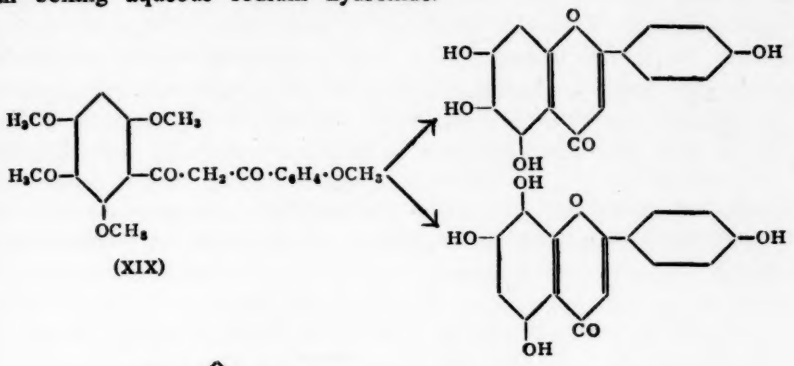
(*b*) 5:6:7-*Combination.*—Though a number of compounds with this arrangement of groups are known, they are not so many as in the previous case.



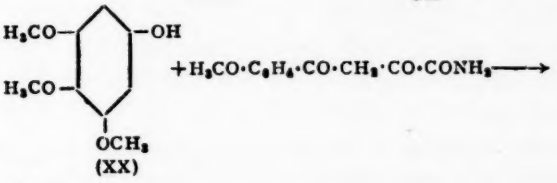
(XVIII)

The two closely related flavones baicalein (XVIII, $R_1 = H$) and scutellarein (XVIII, $R_1 = OH$) occur in the Scutellaria as glucuronides. It is interesting that the former is found in the roots of *Scutellaria baicalensis* whereas the latter occurs in the leaves and flowers. Of the two, scutellarein was the earlier to be discovered and studied. It was isolated by Goldschmiedt and co-workers from the leaves and flowers of *Scutellaria altissima.*[65, 66] It is a tetrahydroxy flavone and it yields on fusion with potash *p*-hydroxybenzoic acid. Methylation with diazomethane gives rise to a trimethyl derivative, and the tetramethyl compound is formed with difficulty using other methods. Consequently it carries two hydroxyl groups in 4′ and 5 positions. It was, therefore, concluded by Goldschmiedt and Zerner[66] that the compound should be either 5:6:7:4′- or 5:7:8:4′-tetrahydroxy flavone. Scutellarein was first synthesised by Bargellini[67] who submitted pentamethoxy dibenzoyl methane (XIX) to the action of hydriodic acid. The reaction could proceed in two ways; but actually scutellarein alone was produced and its constitution was interpreted in the light of certain analogies as 5:6:7:4′-tetrahydroxyflavone. An unequivocal proof of the constitution was given later by Robinson and Schwarzenbach[68] who adopted a novel method of synthesis. By the condensation of anisoyl pyruvamide and antiarol (XX) in the presence of hydrogen chloride 4-carbamyl-5:6:7:4′-tetramethoxyflavylium chloride (XXI) was obtained. Hofmann's reaction converted it into the 4-aminoderivative (XXII). The corresponding free base,

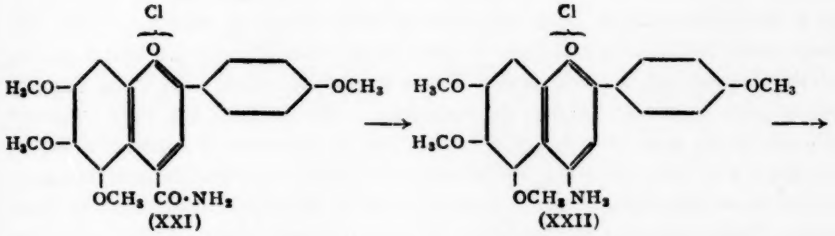
flavone-imine (XXIII) yielded scutellarein tetramethyl ether when treated with boiling aqueous sodium hydroxide.



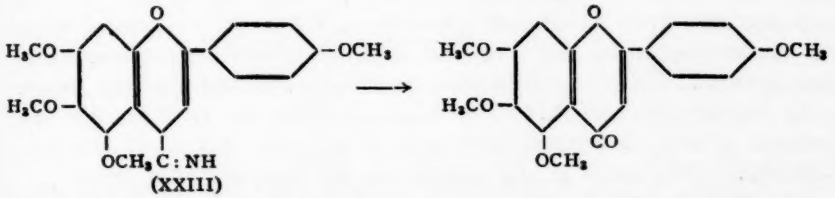
(XIX)



(XX)



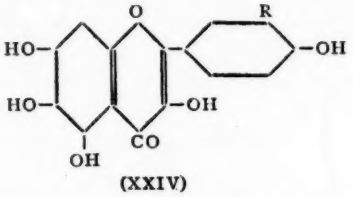
(XXI) (XXII)



(XXIII)

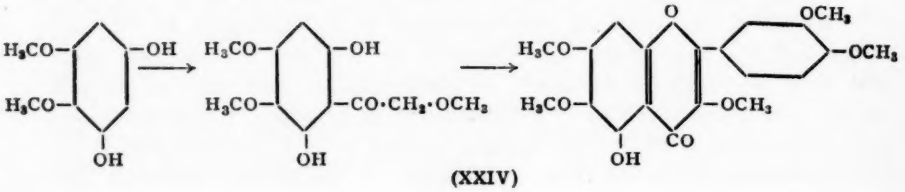
Baicalein was obtained from the roots of *S. baicalensis* by Shibata, Iwata and Nakamura[55] and more recently by Bose and Bhattacharya from the bark of *Oroxylum indicum*.[25] It yields benzoic acid on fusion with alkali. Otherwise it resembles scutellarein in all respects. Consequently its constitution was given by the Japanese authors as 5: 6: 7-trihydroxyflavone. The substance had already been synthesised by Bargellini[67] following the

method adopted by him in the case of scutellarein. Of the two possible isomers only one was again obtained by him and that was the 5:6:7-trihydroxyflavone, baicalein. However a later worker, Hattori, noticed that both the possible isomers 5:6:7- and 5:7:8-trihydroxy (nor-wogonin) flavones were formed. A monomethyl ether of baicalein occurs in nature. It is oroxylin-A isolated by Shah, Mehta and Wheeler[69,25] from the Indian medicinal plant *Oroxylum indicum*. It yielded baicalein on demethylation and on methylation 5:6:7-O-trimethyl baicalein. Thus its relationship with baicalein was established. Since the compound did not give reactions for ortho-hydroxy groups it was concluded that it should be the 6-methyl-ether of baicalein.



(XXIV)

The flavonols belonging to this group are also two being quercetagetin (XXIV, R = OH) and nortangeretin (XXIV, R = H). The former occurs as a glucoside and the latter as its pentamethyl ether tangeretin. There has been some confusion in the use of the correct scientific and common names of the Tagetes species and hence it was originally stated that both *Tagetes erecta* and *T. patula* yielded quercetagetin. The matter has been recently cleared up by Rao and Seshadri[70] who showed that the two species differ in the nature of the colouring matter and that *T. erecta* contains quercetagetin as its monoglucoside, while *T. patula* contains patuletin mostly in the free state. Perkin showed quercetagetin to be a pentahydroxy flavonol yielding protocatechuic acid by alkaline hydrolysis. When its hexamethyl ether was decomposed with alkali veratric acid and tetramethylquercetagetol were obtained. He left unsettled the orientation of the three hydroxyl groups in the benzopyrone part (5:6:7 or 5:7:8) and this was established by the synthesis of both gossypetin and quercetagetin by Baker, Nodzu and Robinson.[3a] The stages in the synthesis of the latter are given below :

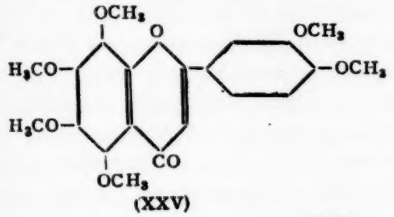


(XXIV)

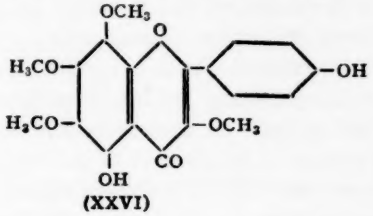
The pyrone ring may close in either of two ways, but the closure took place exclusively in one manner yielding quercetagetin pentamethyl ether. The other alternative would have resulted in the formation of gossypetin derivative and this did not happen.

Tangeretin was isolated by Nelson[71] from the peel oil of tangerine oranges (*Citrus nobilis deliciosa*). By hydrolysis with alcoholic potash anisic acid and a ketone called tangeretol were obtained. The latter resembled quercetagetol tetramethyl ether in all properties and was shown to be identical. Nortangeretin obtained by demethylation exhibited properties very similar to quercetagetin. Consequently Goldsworthy and Robinson[72] represented tangeretin as 3:5:6:7:4′-pentamethoxy flavone and this was supported by its synthesis by the same authors by the condensation of 2:6-dihydroxy-ω:4:5-trimethoxyacetophenone with the anhydride and sodium salt of anisic acid and subsequent methylation of the product.

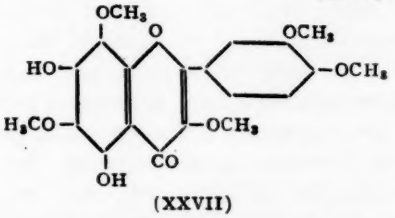
V. Tetrahydroxy benzopyrone group.—Only a few compounds of this group are known and they are very recent discoveries. They do not occur free as such, but are found to be partially or completely methylated.



(XXV)



(XXVI)



(XXVII)

Nobiletin was isolated by Tseng[73] from the peels of the Chinese Mandarin oranges (*Citrus nobilis*). It contained six methoxyl groups and on demethylation yielded a hexahydroxy compound. Veratric acid and actoveratrone were identified among the products of decomposition of the substance with alcoholic potash. The formation of the ketone definitely established that nobiletin was a flavone with two methoxyl groups in the side phenyl nucleus and the other four in the benzopyrone part. Consequently Robinson and Tseng[74] concluded that it is 5:6:7:8:3′:4′-hexamethoxy

flavone (XXV). It has very recently been synthesised by Horii[75] by the condensation of 2-hydroxy-3:4:5:6-tetramethoxy acetophenone with veratroyl chloride and the conversion of the product into the flavone according to the method of Baker and Venkataraman.

Calycopterin is a derivative of a flavonol of this group. It was isolated by Ratnagiriswaran, Sehra and Venkataraman[76] from the copper coloured leaves of *Calycopteris floribunda*. It was also found to be present in the leaves of *Digitalis thapsi* (Spanish digitalis) by Karrer and hence received also the name thapsin.[77] It contained four methoxyl groups and two hydroxyl groups and on fusion with alkali yielded among other products *p*-hydroxy benzoic acid. Hence the nature of the side phenyl nucleus was clear. Calycopteretin, the hexahydroxy compound, produced by demethylation, exhibited the properties of a flavonol. It was unstable to aerial oxidation in alkaline medium and gave a series of colour changes in dilute alkaline solutions. Consequently the location of a hydroxyl in the 3 position and the other four hydroxyl groups in the benzene ring was settled. Thus calycopteretin was 5:6:7:8:4′-pentahydroxy flavonol. The distribution of the methoxyl and hydroxyl groups in calycopterin originally made by the above authors has been recently corrected by Shah, Venkataraman and Virkar.[78] It is stable to aerial oxidation in cold alkaline solution and hence has a methoxyl group in the 3 position. Since calycopterin yields *p*-hydroxybenzoic acid on decomposition with alkali, a hydroxyl is present in the 4 position. The location of the second hydroxyl in 5 position has been made from the following considerations : (1) with diazomethane calycopterin yields a monomethyl ether which gives reactions for a free hydroxyl in the 5 position; (2) this compound could be obtained from the dimethyl ether by selective demethyiation using hydrobromic acid in glacial acetic acid in the cold—selective demethylation of the 5-methoxyl group; (3) in partially methylated flavones the 5-hydroxyl is known to be invariably left free. Calycopterin has been, therefore, given the constitution of 5:4′-dihydroxy-3:6:7:8-tetramethoxyflavone (XXVI).

Erianthin is the next higher member and is one of the highly substituted members of the flavonol group. It has been recently obtained from the plant, *Blumea eriantha* D.C. by Bose and Dutt.[79] It has five methoxyl and two hydroxyl groups. On subjecting it to hydrolytic fission with alkali it yields veratric acid and hence the side phenyl nucleus carries two methoxyl groups in the 3′ and 4′ positions. The other five groups are distributed among the five positions of the benzopyrone part. Since the compound is stable to aerial oxidation in alkaline solution there should be a methoxyl

in the 3 position. Methylation with diazomethane yields only a monomethyl ether and hence the unmethylated hydroxyl is located in the 5 position. Further, erianthin does not respond to tests for the presence of two hydroxyl groups in the ortho- or para-positions. Consequently Bose *et al.* have represented it as 5:7-dihydroxy-3:6:8:3′:4′-pentamethoxy flavone (XXVII).

In the brief review given above it has not been possible to give any detailed account of the methyl ethers of flavones and flavonols, unless they occur as the primary representatives of the type and the corresponding hydroxy compounds do not occur free. Further a number of compounds like gardenin,[80] amarbelin,[81] ginkgetin[82] and sabdaretin[83] which are considered to be definite new compounds but whose constitutions have not yet been definitely established, have been omitted from consideration.

REFERENCES

1. Rangaswami and Seshadri .. *Proc. Ind. Acad. Sci.* (A), 1942, **16**, 129.
2. Wilson .. *J. A. C. S.*, 1939, **61**, 2303.
3. (*a*) Baker, Nodzu and Robinson .. *J. C. S.*, 1929, **74**.
   (*b*) Neelakantam and Seshadri *Proc. Ind. Acad. Sci.* (A), 1937, **5**, 357.
   (*c*) Rao and Seshadri .. *Ibid.*, 1942, **15**, 148.
4. Perkin .. *J. C. S.*, 1913, 657.
5. Rao and Seshadri .. *Curr. Sci.*, 1938, **7**, 227.
6. Pfeiffer .. *Ann.*, 1913, **398**, 137.
7. Bose .. Sir P. C. Ray Commemoration Volume, *J. I. C. S.*, 1933, 65.
8. Quastel .. *Analyst*, 1931, **56**, 311.
9. Shibata and Hattori .. *Br. Chem. Abs.*, 1931, A, 493.
10. Allan and Robinson .. *J. C. S.*, 1924, 2192.
11. Baker .. *Ibid.*, 1933, 1381.
    Mahal and Venkataraman .. *Curr. Sci.*, 1933, **2**, 214.
12. Blount .. *J. C. S.*, 1933, 1528.
13. Robinson and Venkataraman *Ibid.*, 1926, 2344.
14. Allan and Robinson .. *Ibid.*, 1926, 2334.
15. Schmid and Pietsch .. *Monatsh*, 1931, **57**, 305.
16. Brass and Kranz .. *Br. Chem. Abs.*, 1932, **A**, 1256.
17. Schmid and Tadros .. *Ber.*, 1932, **65B**, 1689.
18. Brass and Kranz .. *Ann.* 1932, **499**, 175.
19. Gulati and Venkataraman .. *J. Prakt. Chem.*, 1933, **137**, 53 and 294.
    Badhwar, Kang and Venkataraman .. *J. C. S.*, 1932, 1107.
20. Charlesworth and Robinson.. *Ibid.*, 1933, 268.

21. Limaye .. *Rasayanam*, 1936, **1**, 1937, 119.
22. Manjunath, Seetharamiah and Siddappa .. *Ber.*, 1939, **72B**, 93.
23. Seshadri and Venkateswarlu *Proc. Ind. Acad. Sci.* (A), 1941, **13**, 404; 1943, **17**, 16.
24. Rangaswami and Seshadri .. *Ibid.*, 1939, **9**, 259.
25. Bose and Bhattacharya .. *J. I. C. S.*, 1938, **15**, 311.
26. Nakaoki .. *Br. Chem. Abs.*, 1941, **Aii**, 330.
27. Perkin .. *J. C. S.*, 1900, 423.
    Hattori .. *Ibid., Abstracts*, 1925, **Ai**, 1443.
28. Nakao and Tseng .. *Br. Chem. Abs.*, 1934, A, 820.
29. Tseng .. *Chem. Abs.*, 1935, **29**, 7981.
30. Mahal and Venkataraman .. *J. C. S.*, 1936, 569.
31. Barger and White .. *Biochem. Journal*, 1923, 836.
32. Rao .. *Proc. Ind. Acad. Sci.* (A), 1942, **15**, 123.
33. Nakamura and Hukuti .. *Br. Chem. Abs.*, 1941, **Aii**, 302.
34. Lovecy, Robinson and Sugasawa .. .. *J. C. S.*, 1930, 817.
35. Oesterle and Wander .. *Helv. Chim. Acta*, 1925, **8**, 519.
36. Anderson and Perkin .. *J. C. S.*, 1931, 2624.
37. ——— .. *Canad. J. Res.*, 1932, **7**, 285.
38. Gulati and Venkataraman .. *J. C. S.*, 1933, 942 and 1644.
39. Kalff and Robinson .. *Ibid.*, 1925, 1968.
40. (*a*) Kalff and Robinson .. *Ibid.*, 1925, 181.
    (*b*) Heap and Robinson .. *Ibid.*, 1926, 2336.
    (*c*) Chavan and Robinson .. *Ibid.*, 1933, 368.
41. Kimura and Hoshi .. *Chem. Abs.*, 1937, **31**, 6654.
42. Sando .. *J. Biol. Chem.*, 1932, **94**. 675.
43. Robinson and Shinoda .. *J. C. S.*, 1925, 1980.
44. Akai .. *Br. Chem. Abs.*, 1936, **A**, 710.
    ——— and Matsukawa .. *Ibid.*, 1937, **Aii**, 7.
45. Desai and Ahmad .. *Proc. Nat. Inst. Sci.*, India, 1939, **5**, 261.
46. Charaux .. *J. C. S., Abstracts*, 1925, **Ai**, 823.
47. Robinson and Venkataraman *J. C. S.*, 1929, 61.
48. Nagai and Hattori .. *Br. Chem. Abs.*, 1930, A, 784.
49. Baker .. *J. C. S.*, 1939, 956.
50. ———, Brown and Scott .. *Ibid.*, 1939, 1922.
51. Horii .. *Br. Chem. Abs.*, 1940, **Aii**, 231.
52. Nakazawa .. *Chem. Abs.*, 1940, **34**, 1017.
53. Neelakantam, Rao and Seshadri .. *Proc. Ind. Acad. Sci.* (A), 1941, **14**, 105.
54. Rao and Seshadri .. *Ibid.*, 1941, **14**, 643.
55. Shibata, Iwata and Nakamura *J. C. S., Abstracts*, 1923, **Ai**, 592.
56. Hattori .. *Br. Chem. Abs.*, 1931, A, 493.
57. ——— .. *Br. Chem. Abs.*, 1932, **A**, **64**.

58. Shah, Mehta and Wheeler .. *J. C. S.*, 1938, 1555 ; *Curr. Sci.*, 1938, **6**, 503.
59. Neelakantam, Rao and Seshadri .. *Proc. Ind. Acad. Sci.*, (A), 1943, **17**, 26.
60. Rao and Reddi .. *Ibid.*, 1940, **12**, 372.
61. Goldsworthy and Robinson *J. C. S.*, 1938, 56.
62. Bose and Bose .. *J. I. C. S.*, 1939, **16**, 183.
63. Perkin .. *J. C. S.*, 1909, 1855.
64. Rao .. *Curr. Sci.*, 1942, **11**, 360.
65. Goldschmiedt and Molisch .. *Monatsh*, 1901, **22**, 679.
66. ——— and Zerner .. *Ibid.*, 1910, **31**, 439.
67. Bargellini .. *Gazetta*, 1919, **49**, **ii**, 47.
68. Robinson and Schwarzenbach .. *J. C. S.*, 1930, 822.
69. Shah, Mehta and Wheeler .. *Ibid.*, 1936, 591.
70. Rao and Seshadri .. *Proc. Ind. Acad. Sci.* (A), 1941, **14**, 289.
71. Nelson .. *J. A. C. S.*, 1934, **56**, 1392.
72. Goldsworthy and Robinson .. *J. C. S.*, 1937, 46.
73. Tseng .. *Ibid.*, 1938, 1003.
74. Robinson and Tseng .. *Ibid.*, 1938, 1004.
75. Horii .. *Br. Chem. Abs.*, 1941, **Aii**, 331.
76. Ratnagiriswaran, Sehra and Venkataraman .. *Biochem. J.*, 1934, **28**, 1964.
77. Karrer .. *Helv. Chim. Acta*, 1934, **17**, 1560.
——— and Venkataraman *Nature*, 1935, **135**, 878.
78. Shah, Venkataraman and Virkar .. *J. I. C. S.*, 1942, **19**, 135.
79. Bose and Dutt .. *Ibid.*, 1940, **17**, 45.
80. ——— and Nath .. *Ibid.*, 1938, **15**, 139.
81. Agarwal .. *Ibid.*, 1936, **13**, 531.
82. Furukawa .. *Br. Chem. Abs.*, 1933, A, 1168.
Baker and Simmonds .. *J. C. S.*, 1940, 1370.
83. Rao and Seshadri .. *Proc. Ind. Acad. Sci.* (A), 1942, **16**, 323.

338-43 Printed at The Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent, and Published by The Indian Academy of Sciences, Bangalore.